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In silico Design, and in vivo evaluation of synthesized phenyl isoserine derivative and used as anticytokine storm

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Abstract

Cytokine storm is several disorders of immune dysregulation characterized by constitutional symptoms, systemic inflammation, and multiorgan dysfunction that can lead to multiorgan failure if inadequately treated, 1,2,3-Triazoles are one of the most important nitrogen-containing five-membered heterocycles and have a wide range of applications in pharmaceuticals, supramolecular chemistry, organic synthesis, chemical biology and industry.In the current work , the synthesized compounds (1d and 2d) were screened for their anticytokine storm by assay of IL-1b and IL-6 in vivo, confirmations and characterization of the chemical structures related to these compounds were performed using 1H-NMR spectroscopy, FT-IR spectroscopy, and some physicochemical properties such as melting points. Docking study of the final synthesized compounds gave evidence about the affinity of these compounds significantly inhibited IL in comparison to positive groups

Keywords: Anti-cytokine storm; N-benzyle phenylisoserine; molecular docking 1,2,3-Triazole

1. Introduction

Cytokines are short-lived (15–20 kDa) proteins that play a role in autocrine, paracrine, and endocrine signaling. Cytokines coordinate the immune system's development and activity[1], and they can have a wide range of impacts on many different cells throughout the body[2]. Cytokines can act systemically throughout the host in an endocrine manner, paracrinely on only nearby cells in a paracrine manner, or autocrinely on the cell that produced them. The fact that cytokines act only through specific cytokine receptors on cells demonstrates how important these molecules are to an effective and non-harmful immune response. Cytokines are separated into groups depending on their receptor types and their mechanism of action in the immune system, such as proinflammatory, anti-inflammatory, or adaptive. [3] Many cytokines have additional roles not represented in their pro-inflammatory, anti-inflammatory, or adaptive classifications. As a result, their immunoregulatory functions are characterized to simply as "a main function" or "an important function," rather than "the main function" or "the most essential function." This stringent cytokine classification of 'pro-inflammatory,' 'anti-inflammatory,' and 'adaptive' has not been applied. The IL-1, IL-6, IL-17, interferon, and TNF families are the most common pro-inflammatory cytokines. The IL-1 family of cytokines is required for the start of the inflammatory cascade [4], while the IL-6 family of cytokines has both immunoregulatory and other systemic effects. [5] The term "cytokine storm" refers to a group of immune dysregulation illnesses marked by constitutional symptoms, systemic inflammation, and multiorgan dysfunction, which can lead to multiorgan failure if not treated properly. Depending on the etiology and treatments used, the onset and duration of cytokine storm varies.[6]

1,2,3-Triazoles are one of the most important nitrogen-containing five-membered heterocycles in medicines, supramolecular chemistry, organic synthesis, chemical biology, and industry. [7] High

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chemical stability (generally inert to acidic or basic hydrolysis as well as oxidizing and reducing conditions even at high temperatures), aromatic character, strong dipole moment (4.8–5.6 Debye), and hydrogen bonding capacity are only a few of the advantages of 1,2,3-triazoles[8]. The substituted 1,2,3-triazole motif is structurally similar to the amide bond, approximating an E or Z amide bond, thanks to these striking properties. There are many well-known medical compounds with a 1,2,3-triazole core on the market, such as the anticonvulsant medicine Rufinamide, the broad-spectrum cephalosporin antibiotic cefatrizine, the anticancer drug carboxyamidotriazole, and the -lactum antibiotic tazobactam, among others..[9].

(2R,3S)-N-Benzoyl-3-phenylisoserine is a structural component of the antimicrotubular drug paclitaxel (Taxol). Paclitaxel 1 (Figure 1) is a complex taxane diterpenoid initially isolated[10]

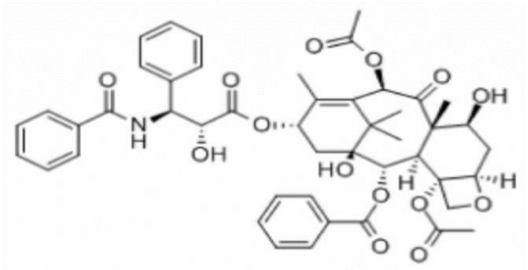


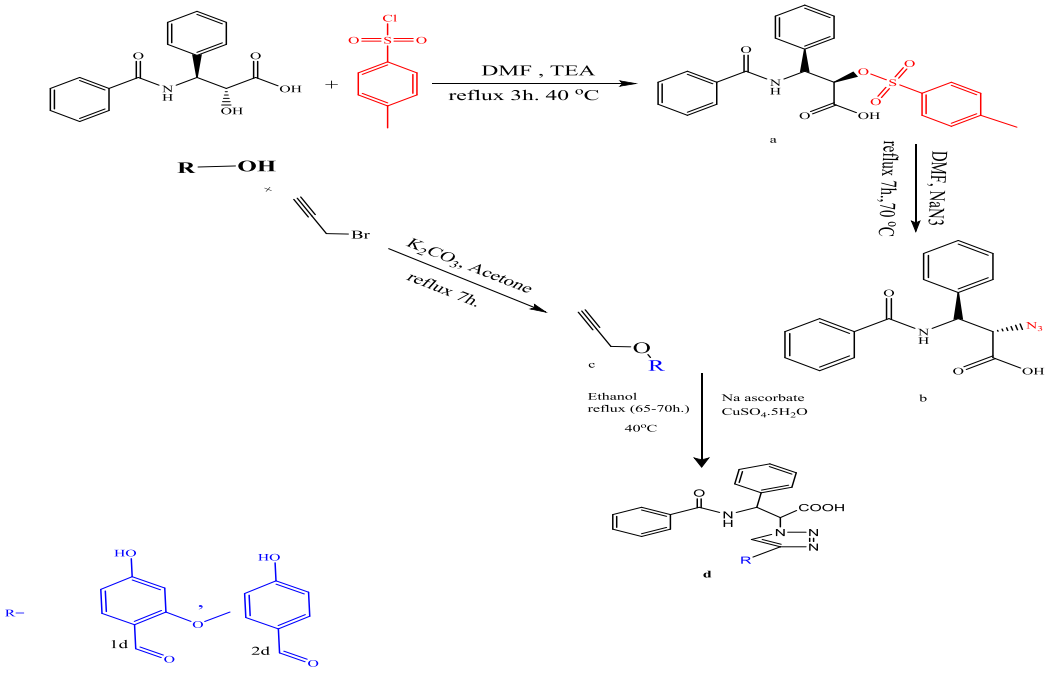
Figure (1): Chemical structures of paclitaxel 1

2. Experimental:

All of the reagents and anhydrous solvents were analytical grade and came from the same source (Germany, China, India, Spain, USA). Electro-thermal melting point apparatus capillary tube method was used to determine melting points. The faculty of science at Karbala University used an FTIR spectrophotometer to determine the infrared spectrum. KBr discs were used to determine the spectrum. Mashad University in Iran conducted the 1HNMR spectrum. The solvent was DMSO, and the instrument was a Bruker 500MHz-Avanc.

Chemical Synthesis

The synthesis of the target compounds (a-d) and their intermediates were achieved following procedures illustrated in scheme (1).



Scheme 1: Synthesis of intermediates and target compounds.

Synthesis of compound (a) 3-benzamido-3-phenyl-2-(tosyloxy)propanoic acid

N-benzoyl (2R,3S)-2-phenylisoserine (6 g, 0.02 mol) in (25 ml) dimethyformamide (DMF) was placed in a round bottomed flask 100 ml in size with triethylamine (TEA) (2.8ml, 0.02 mol), fitted with a stirring bar and stirred. After 15 min, (4 g, 0.02 mol) of p-toluenesulfonyl chloride was added and stirring for 2h. the mixture was refluxed for 4 h. in 40^{o}C (until the reaction was completed as checked by TLC.

The contents of the flask were cooled under room temperature, then filtered, washed and dried to obtain the precipitate of 3-benzamido-3-phenyl-2-(tosyloxy) propanoic acid (compound a).

FT-IR(KBr, cm^{-1}): 3063(Broad (O-H) stretching of carboxylic acid), 1361((O=S=O) symmetric & asymmetric vibration), 1716(C=O stretching vibration of amide), 3344(N-H stretching vibration of secondary amide).

1H NMR (301 MHz, DMSO-d6) δ 8.64 (d, J = 8.9 Hz, 1H), 7.94 – 7.83 (m, 2H), 7.65 – 7.40 (m, 5H), 7.41 – 7.29 (m, 2H), 7.33 – 7.21 (m, 1H), 5.51 (dd, J = 8.9, 4.2 Hz, 1H), 4.43 (d, J = 4.3 Hz, 1H), 2.56 (s, 3H).

Synthesis of compound (b): 2-azido-3-benzamido-3-phenylpropanoic acid

Compound (a) (3.5g, 0.011 mol) was dissolved in 25 ml DMF, stirred for 15 min, then (0.71g, 0.011 mol) of sodium azide was added to the solution and refluxed for 7 h. in 60^{o}c (until the reaction was completed as checked by TLC) After completion of reflux, the solvent was evaporated by rotary evaporator, and the product was filtered, and collected as compound (b).

FT-IR(KBr, cm^{-1}): 3390(Broad (O-H) stretching of carboxylic acid), 2110((N≡N) stretching vibration), 1739(C=O stretching vibration of amide), 3306(N-H stretching vibration of secondary amide).

1H NMR (301 MHz, DMSO-d6) δ 9.27 (d, J = 8.1 Hz, 6H), 8.45 (s, 1H), 7.83 (dt, J = 6.8, 1.6 Hz, 13H), 7.63 – 7.43 (m, 21H), 7.40 – 7.32 (m, 13H), 7.32 – 7.11 (m, 22H), 5.26 (dd, J = 8.1, 3.9 Hz, 7H), 3.94 (d, J = 4.0 Hz, 8H)

Synthesis of compound (1C):4-ethynyloxy -3-methoxybenzaldyhyde:

We take 1 mole of (4-hydroxy-3-methoxybenzaldyhyde and 4-hydroxy benzaldyhyde) and dissolve it with acetone, then add 1 mole of potassium carbonate, stir, then add 1 mole of Probagel Bromide (Probagel is added drop by drop and at a low temperature) the mixture was refluxed for 7 h. in 50^{o}C (until the reaction was completed as checked by TLC.

The contents of the flask were cooled under room temperature, then filtered, washed and dried to obtain the precipitate of compounds.

FT-IR(KBr,cm^{-1}): 2111(C≡C) stretching vibration), 3244(≡C-H) stretching vibration), 3078((C-H) aromatic stretching vibration), 1218(C-O-C) stretching vibration) of 4-ethynyloxy -3-methoxybenzaldyhyde

FT-IR(KBr,cm^{-1}): 2113 (C≡C) stretching vibration), 3200(≡C-H) stretching vibration), 3064(C-H) aromatic stretching vibration), 1241(C-O-C) stretching vibration) of 1-(ethynyloxy)-4-hydroxybenzaldyhyde.

Synthesis of compound (1d):

We take (1mole, 0.66 g) of 2-azido-3-benzamido-3-phenylpropanoic acid and dissolve it in methanol and stir the solution for 10 min at the same time we dissolve (1 mole, 0.026 g) of CuSO4.5H2O and (1mole ,0.042 g) of sodium ascorbate in methanol and leave the reaction with stirring for 10 min and then Add to the last a methanol solution containing 1-(ethynyloxy)-4-nitrobenzen by taking (1 mole, 0.57 g) of it and dissolving it with methanol, mixing the mixture well and placing it in the reflex device for 70 h. In 40 ^{o}c.

FT-IR(KBr, cm^{-1}): 1222((N-N=N) stretching vibration), 1739((C=O) stretching vibration of carboxylic acid), 1٦43((C=O) stretching vibration of amide), 2928((C-H) aromatic stretching vibration), 3248(Broad (O-H) stretching of carboxylic acid), 3360(N-H stretching vibration of secondary amide), 1010((C-O-C) stretching vibration).

1H-NMR (300 MHz, DMSO-d6) δ 9.87 (s, 1H, -CHO), 8.75 (s, 1H, triazole ring), 7.96-7.09(aromatic protons), 5.94(d, J = 4.0 Hz, 1H, CH-triazole ring), 5.45 (d, J = 8.1, 3.9 Hz, 1H, Ph- CH-), 3.52(s, 3H, -O-CH3).

Synthesis of compound (2d):

We take (1mole, 0.6 g) of 1-(ethynyloxy)-4-hydroxybenzaldyhyde and dissolve it in methanol and stir the solution for 10 min at the same time we dissolve (1 mole, 0.024 g) of CuSO4.5H2O and (1mole ,0.038 g) of sodium ascorbate in methanol and leave the reaction with stirring for 10 min and then Add to the last a methanol solution containing 1-(ethynyloxy)-4-nitrobenzen by taking (1 mole, 0.32 g) of it and dissolving it with methanol, mixing the mixture well and placing it in the reflex device for 55 h. In 40 ^{o}c.

FT-IR(KBr, cm^{-1}): 1222((N-N=N) stretching vibration), 1739((C=O) stretching vibration of carboxylic acid), 1٦93((C=O) stretching vibration of amide), 2974((C-H) aromatic stretching vibration), 3009(Broad (O-H) stretching of carboxylic acid),

3282(N-H stretching vibration of secondary amide), 1157((C-O-C) stretching vibration).
1H-NMR (300 MHz, DMSO-d6) δ 9.27 (s, 1H, CHO), 8.45 (s, 1H, triazole ring), 7.84-7.16 (aromatic protons), 5.26 (dd, J = 8.1, 3.9 Hz, 1H, Ph- CH-), 4.53 (s, 2H, -O-CH2-), 3.94 (d, J = 4.0 Hz, 1H, CH-triazole ring).

Table (1): The characterization and physical data of the synthesized compounds

Compound and intermediates	Chemical formula	Molecular weight	Description	Melting point ^{0}C
a	$C_{23}H_{20}O_6S_1N_1$	439	White powder	60-65
b	$C_{16}H_{14}O_3N_4$	327	Faint yellow	50-57
1c	$C_{11}H_{10}O_3$	190.20	Yellow solid	68-70
2c	$C_{10}H_8O_2$	160.17	Yellow powder	79-81
1d	$C_{27}H_{24}N_4O_5$	484.50	Yellowish gray	177-180
2d	$C_{26}H_{22}N_4O_4$	454.48	bright yellow	175-185

3. Experimental Animal

Fifty male Swiss albino mice weighing 25 – 30g each had been purchased from the college of pharmacy- Al-Nahrain University. All animal handling and experimental procedures have been performed in strict accordance with the recommendations in the guide for the care and use of laboratory animals of the animal ethics committee AL-Nahrain University/ College of pharmacy. Animals were left free in the animal care facility of Al-Nahrain University College of pharmacy a 12-hour light/dark cycle. Regular rodent chow and water were provided ad libitum. The room was well ventilated with 100% fresh air. The temperature was set to standard levels of 23 ± 2 °.

Samples preparation

All chemical compounds prepared as fresh stock solution equivalent to 200mg/ kg by dissolving it in DMSO (1%) and diluted gradually with distilled water, while

Cytokine storm induction

5 mg/kg Lipopolysaccharide (LPS) (Escherichia coli, serotype 0111: B4 prepared as stock 1 mg/ml according to the manufacturer's instructions/Sigma-Aldrich [11]; 300µl injected through intra-peritoneum.

Interleukin-6 detection

Fifty Swiss albino mice, weighing 25 to 30 g on average, were chosen at random and divided into five groups of ten mice. Group A receives nothing but the vehicle used to dissolve the chemical compounds, while Group B receives only LPS and is considered a positive control. 200 milligrams per kilogram (1d,2d). Blood was taken from the jugular vein after 24 hours. To allow the blood sample to coagulate, it was held at room temperature for 15 minutes. After that, a tube centrifuge was used to spin the test tube containing the clotted blood sample at 3000 r/min for 10 minutes to separate the serum from the clotted blood. The clear serum was then carefully aspirated with a syringe and needle and stored in a clean sample bottle at -20 °C ,subsequent thawing for the quantitative determination of interleukine-6 by enzyme-linked immunosorbent assay (ELISA) method.

Interleukin-1b detection

Fifty Swiss albino mice of average weight between 25 to 30 g, which were selected randomly and then divided into five groups of ten mice. Group A, Group B, Group C, Group D and Group E. group A receive nothing but the vehicle used to dissolve the chemical compounds group B receive LPS only and consider as positive control. 200mg/kg with compound (1d,2d). After 24 blood was collected from jugular vein. The blood sample was kept at room temperature for 15 min to clot. Afterward, the test tube containing the clotted blood sample was centrifuged at 3000 r/min for 10 min using a tube centrifuge to enable complete separation of the serum from the clotted blood. The clear serum was then carefully aspirated with a syringe and needle and stored in a clean sample bottle at -20 °C ,subsequent thawing for the quantitative determination of interleukine-1b by enzyme-linked immunosorbent assay (ELISA) method.

4.Docking Study:

The molecular docking technique has become a critical tool in the drug development process. We can assess the interaction between the protein and the ligand in the study using this method.
Protein and ligand preparation are both part of the docking process. Protonation of three-dimensional structure in AutoDock Vina under the supervision of

pyRX- pythonprescription version 0.8; software of Discovery studio visualizer (version 21.1.0); and energy minimization were all part of the ligands preparation process. The protein is a type of protein. Interlukine 1b (PDB ID: 2MIB) and interlukine 6 (PDB ID: 2l3y) crystal structures were retrieved from the protein data bank, and all water molecules and metals were deleted, as well as hydrogen atoms.

5. RESULTS AND DISCUSSION:

5.1.chemistry :

Mechanism of synthesis for compounds (1d and 2d):

Step 1: formation of azide:

To summarize, the proposed 3-benzamido-3-phenyl-2-(tosyloxy)propanoic acid is obtained by a nucleophilic attack of the hydroxide [the unshared pair of electrons on the oxygen] on the corresponding electrophile in toluenesulfonyl chloride, followed by a nucleophilic attack of the azide ion on the carbon atom of 3-benzamido-3-phenyl-2-

Step 2: formation of (prop-2-yn-1-yloxy) derivatives

The derivatives are made using the Williamson reaction, which uses anhydrous potassium bicarbonate (K2CO3) as a base to extract the proton of the hydroxyl group[12], as it provides a good nucleophilic (phenoxied ion) to attack the carbon atom associated with the halogen present in propagel bromide. As a result, acetone is one of the best solvents for this type of reaction.

Step 3: formation of phenyl isoserine derivatives:

It can be seen from , the mechanism of this step that the initial formation of a copper sulfate. Subsequent coordination of an organic azide is followed by cycloaddition to form a 6-membered ring which undergoes ring contraction to form a copper triazolide intermediate. Protonolysis then results in elimination of the product triazole.[13]

The final synthesized derivatives were achieved in a pretty high yield, and their physicochemical attributes are listed in tables (1). The melting points of all the produced compounds were evaluated and recorded in this investigation.

The predicted log P (n-octanol / water partition coefficient) parameters, the H-bond donors, and the H-bond acceptors all show extremely good acceptance for the Lipinski rule of five (also known as Pfizer's rule of five) based on the observed physical and chemical properties of these compounds.[14]:

The following investigations were carried out on each of the synthesized compounds separately in order to compare their structures to theoretical ones and to track the reaction pathways to obtain pure products. The results of these tests, as well as their interpretations, are listed below.

Infrared spectroscopy is a common technique for drug analysis and characterisation since it is fast, accurate, and non-destructive.[15]

The purpose of using FT-IR in this study was to identify and confirm the structures of the synthesized compounds based on the appearance and disappearance of a characteristic band in the spectrum[16].

The FT-IR spectra of the synthesized compounds showed characteristic bands of absorbance, which were in consistence with proposed structure of these compounds.

The values of the characteristic bands of these spectra were discussed according to the literature survey of similar compounds and references [17]

The melting points of the synthesized compounds were measured using the melting point test apparatus, and the results were documented in a table (1). The melting point of a chemical compound is one of the physical criteria that is commonly used for identification and purity detection. [18].

For the synthesized compounds, the sharp and characteristic melting point range of values observed gives a fair indication of purity and characterisation.

1H-NMR analysis was used to identify the final derivatives. The spectra were recorded in DMSO-d6 (deuterated dimethyl sulfoxide) solvent. Values of characteristic chemical shifts have been discussed as expressed by references books and according to literature survey of similar compounds.[19]

5.2.Pharmacology

anticytokine study:

The purpose of this part of the study was to evaluate the biological activity of the chemical compounds prepared in this thesis 300 µl were injected through the peritoneum with the compounds in addition to the formation of the cytokine storm by dosing it with LPS.

The anti-cytokine storm activity of the tested compounds has been evaluated in comparison with their vehicle (negative control) and (positive control). The intraperitoneal injection of tested the compound (C, D)(100mg/kg) produced significant difference between compounds and positive control $p < 0.05$ in IL-6 and IL-1B show in figure (1) -(4) but group G significantly inhibited IL6 in comparison to other treated groups.

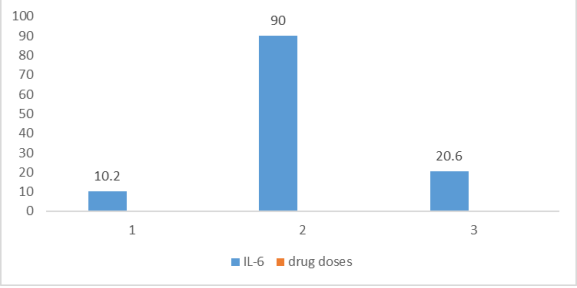


Figure (1): statistical result of IL-6 with compound 1d (1=GPA ,2=GPB, 3=GPC)

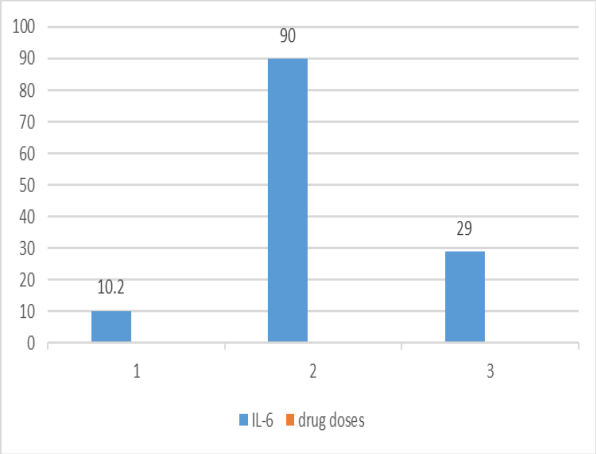


Figure (2): statistical result of IL-6 with compound 2d (1=GPA ,2=GPB, 3=GPD)

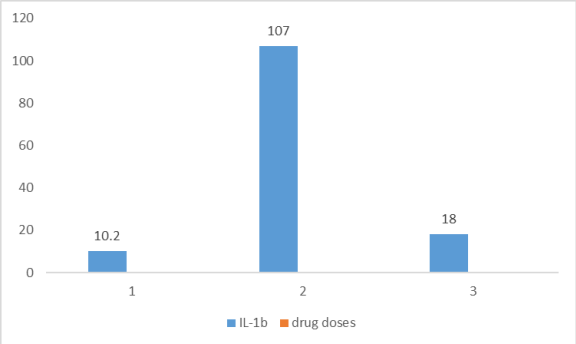


Figure (3): statistical result of IL-Ib with compound 1d (1=GPA ,2=GPB, 3=GPC)

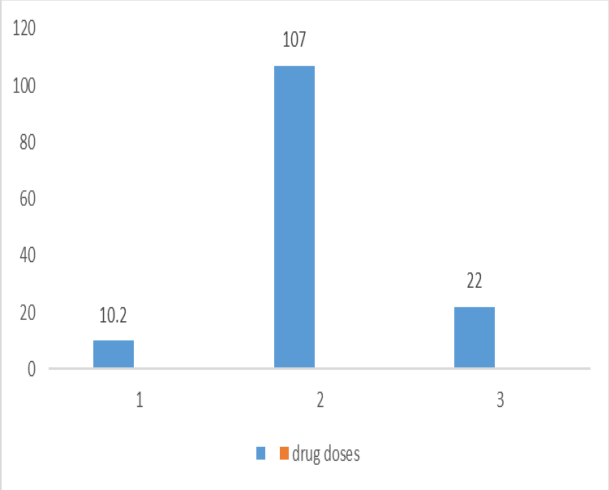


Figure (4): statistical result of IL-Ib with compound 2d (1=GPA ,2=GPB, 3=GPD)

5.3. Docking Study

The docking studies were done byby pyRX-pythonprescription the software of Discovery studio visualizer. the interlukine 1b, 6 (IL1b,IL6) (PDB ID: 2MIB and 2l3y) is the target protein. with the (1d,2d) compounds.The docking of the chemical structures Gatifloxacin, 1d and 2d as shown in figures (5–8)

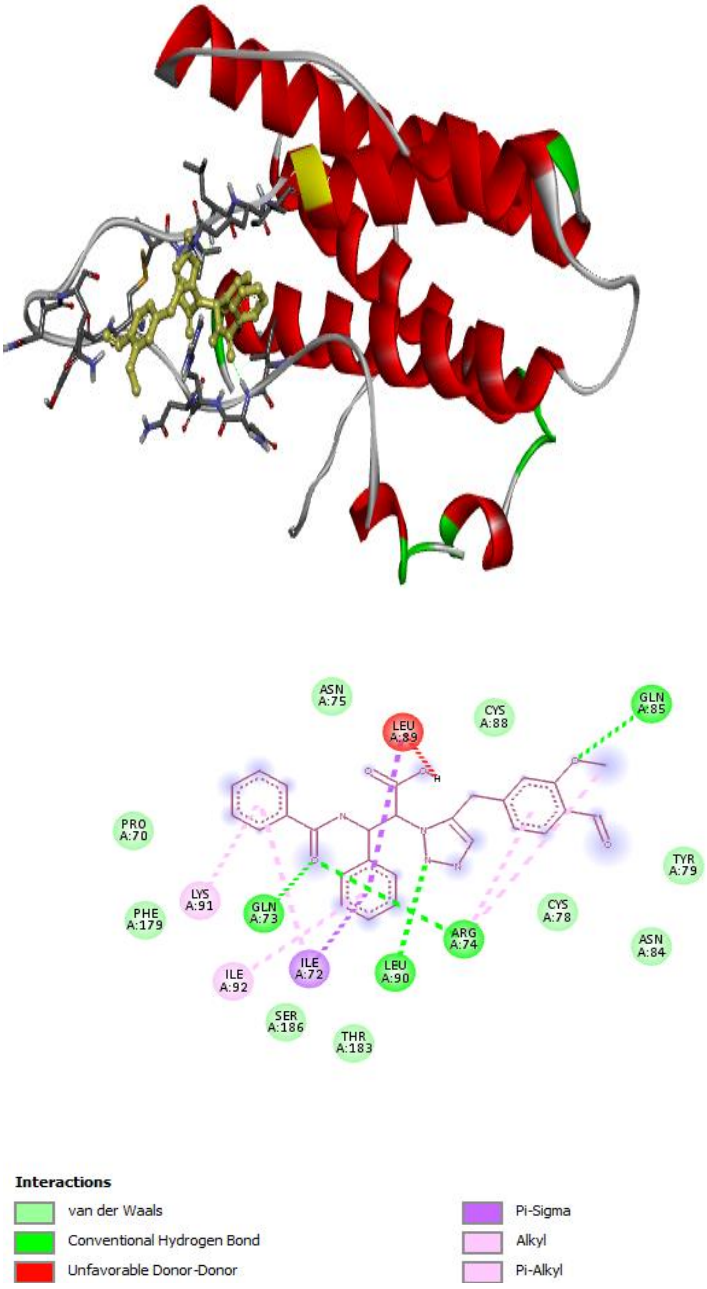


Figure (5): Crystal structure of compound 1d with IL-6 (PDB entry: 2L3Y).[4d: Ball and stick style, amino acid residues in a capped stick style]

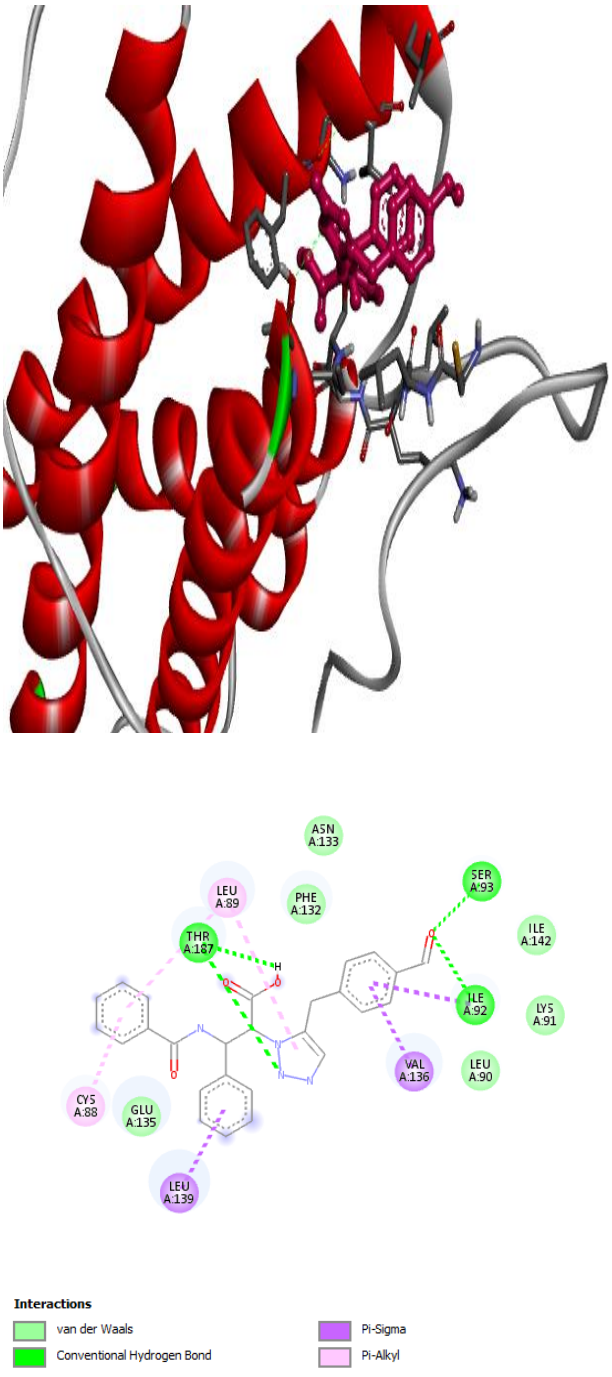


Figure (6): Crystal structure of compound 2d with IL-6 (PDB entry: 2L3Y).[5d: Ball and stick style, amino acid residues in a capped stick style]

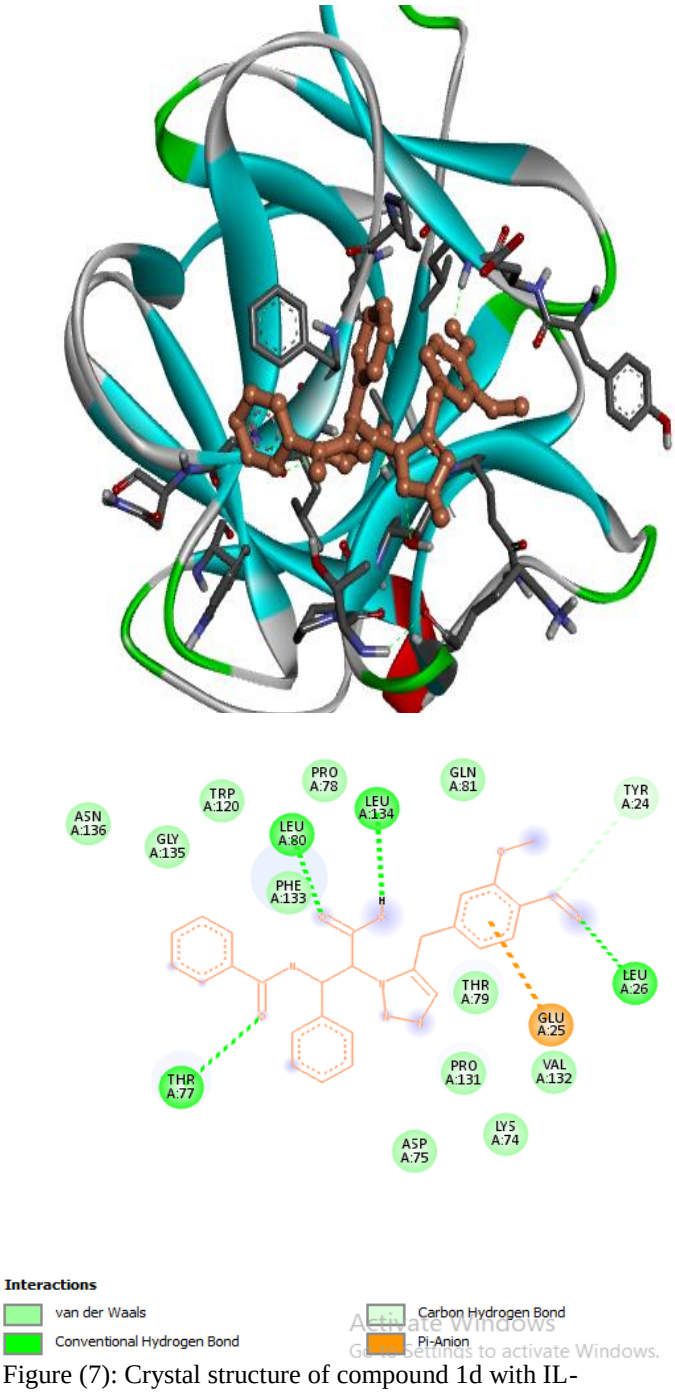


Figure (7): Crystal structure of compound 1d with IL-1b (PDB entry: 2MIB).[4d: Ball and stick style, amino acid residues in a capped stick style]

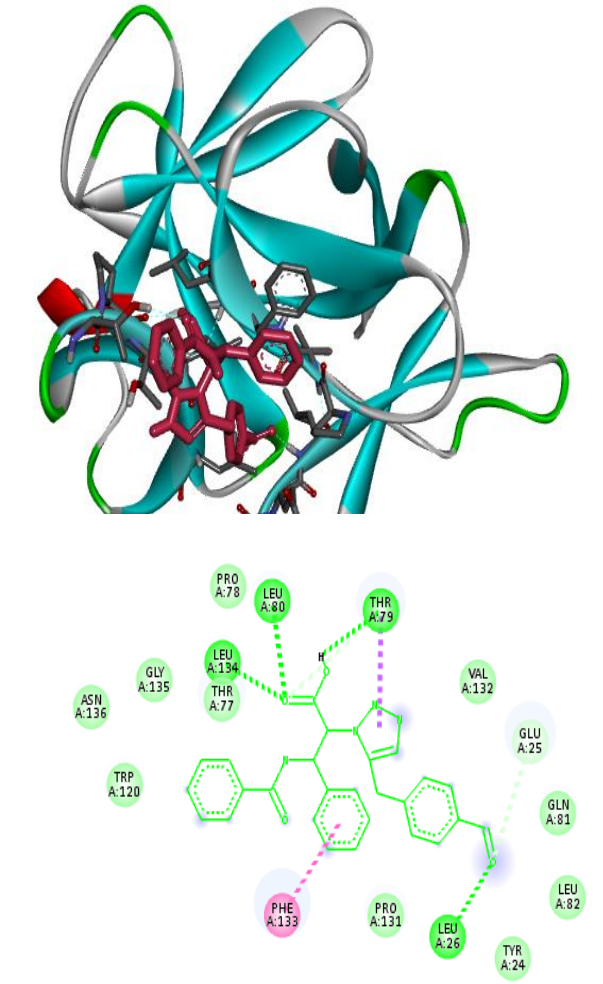


Figure (8): Crystal structure of compound 2d with IL-1b (PDB entry: 2MIB).[5d: Ball and stick style, amino acid residues in a capped stick style]

6. Conclusions:

The antictokine storm study showed that the compound 1d and 2d act as inhibited to the cytokine storm compared with the positive control in the study.

7. Conflicts of interest

"There are no conflicts to declare".

8. Acknowledgments:

Special thanks to alkafyl university/Dr.Ali al thabhwy and to my family and all friends for enabling us to perform this study.

8.Referances

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